

## Reduction of fusarium wilt of hydroponically grown basil by *Fusarium oxysporum* strain CS-20

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### Abstract

Basil seedlings were drenched with water or *Fusarium oxysporum* strain CS-20. The following week, plants were placed in hydroponic troughs with a circulating nutrient solution. Since 2% of the seed was naturally infested with *F. oxysporum* f. sp. *basilici*, pathogen inoculum was not applied. After 7 weeks, there were significantly fewer dead stems in plants treated with strain CS-20 than in the control treatment. Laboratory experiments were conducted to determine if a different type of seeding medium could retard dispersal of the pathogen. Oasis is a rigid, open-celled foam used as a medium for seeding and for rooting cuttings. Oasis cubes (cubes physically attached to each other within each tray) and wedges (wedges physically separated within each tray) were seeded with basil. The cube or wedge nearest to the center was inoculated with  $10^5$  propagules of the pathogen or water. After 1 and 2 weeks, half of the cubes and wedges in each of the three trays were destructively sampled. Different trays were used for the 1- and 2-week samplings. For treatments inoculated with the pathogen, the number of colony forming units (cfu) recovered decreased significantly as the distance from the inoculation site increased. Significantly more cfu were recovered from cubes than wedges when both planting media were inoculated with the pathogen, indicating that the cubes were more conducive to pathogen dispersal than the wedges. In the cubes, the pathogen was recovered 13 cm away from the inoculation site at populations significantly higher than the background levels (up to  $10^6$  propagules/cube). Published by Elsevier Science Ltd.

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### 1. Introduction

The soil-inhabiting fungus *Fusarium oxysporum* Schlechtend.:Fr. causes wilt diseases and root and crown rots on plants in at least 147 different genera in the United States (Farr et al., 1989). *Fusarium* on basil was first found in the US in 1990 (Wick and Haviland, 1992). Isolates of the pathogen collected from seed and diseased plants in the US were all in the same vegetative compatibility group (Elmer et al., 1994). *F. oxysporum* f. sp. *basilici* can be soil, seed or, after sporulating on plant stems, airborne (Gamliel et al., 1996; Rekah et al., 2000).

Several nonpathogenic species of *Fusarium* have been reported to control Fusarium wilt on various crops (Alabouvette et al., 1998). *F. oxysporum* strain CS-20

has been reported to control Fusarium wilt on tomato, muskmelon and watermelon (Larkin and Fravel, 1998, 1999b,c). Strain CS-20 may be different from other strains of *Fusarium* used as biological control agents, because CS-20 works primarily through induced resistance rather than competition (Larkin and Fravel, 1999a). Thus, simply because CS-20 controls Fusarium wilt on one type of plant, it does not guarantee that it will control wilt on another type of plant.

This research was initiated when we were contacted by a grower with a hydroponic basil facility experiencing severe economic losses due to Fusarium wilt. Stem isolations and subsequent pathogenicity tests on basil confirmed *F. oxysporum* f. sp. *basilici* as the causal agent (data not shown). The nutrient solution that circulated through the hydroponic system passed through a uv sterilizer on each pass through the greenhouse. Samples of the circulating nutrient solution collected on two dates indicated that the uv sterilizer was working properly and no *Fusarium* could be recovered from the

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nutrient solution exiting the uv sterilizer. Samples of the circulating nutrient solution collected at other points throughout the facility contained background levels of *Fusarium* that ranged from 0 to 53 cfu/ml (colony-forming units—cfu), with a mean of 25 cfu/ml. Nevertheless, visual observation revealed that the disease appeared to occur in an aggregate distribution across several hydroponic troughs, suggesting the spread of the disease from a point source. This distribution could arise from sporulation of the pathogen on the stems and/or from the spread of the disease, while in the seedling stage wherein plants are in physical contact before they are separated and placed in the hydroponic system. Because of the size of the greenhouse and the fact that new basil plants were seeded each week, it was possible to observe plants and disease at all stages of development. Sporulation of *Fusarium* on the stems occurred very near to harvest of the plants, and thus, we hypothesized that stem sporulation had little effect on the yield of the current crop. We did not observe the foliar infections as reported by Rekah et al. (2000). Thus, we hypothesized that early infections were important to the spread of the disease in this production system and we chose to investigate spread of *Fusarium* through the seeding medium. The objectives of this research were to: (i) determine if *F. oxysporum* strain CS-20 has a potential in reducing losses to *Fusarium* wilt of basil, (ii) assess the capacity of *F. oxysporum* f. sp. *basilici* to spread through the seeding medium, and (iii) determine if a different seeding medium could reduce the spread of the pathogen from a point source.

## 2. Materials and methods

### 2.1. Fungi

*F. oxysporum* f. sp. *basilici* strain Fo-B1, isolated from basil seed, and *F. oxysporum* strain CS-20 were maintained on potato dextrose agar (Difco, Detroit, MI). Inoculum was produced in a aqueous suspension of 1% (w:v) ground soy hull fiber as described previously (Dietfiber soy fiber; Lauhoff Grain Co., Danville, IL) (Hebbbar et al., 1996; Larkin and Fravel, 1999a).

### 2.2. Biological control experiments

Placing the grower's seed onto Komada's medium (Komada, 1975) revealed that 2% of the seed was infested with the pathogen. Fifteen basil seeds (large leaf Italian) were planted in each cell of Oasis Rootcubes ( $2 \times 2 \times 3.5$  cm (tall); 276 (=  $12 \times 23$ ) contiguous cubes/tray; (BFG Supply, Burton, OH)) at a commercial hydroponic facility. Oasis is a rigid, open-celled foam

used for seeding and rooting cuttings. One week after seeding, each of the five trays was drenched with 900 ml of  $10^6$  propagules (78% microconidia + 22% chlamydospores) of CS-20 or water (Fig. 1). There were five replicate trays for each treatment. The following week, cubes were separated and placed in troughs (PVC pipe with holes) of nutrient solution that circulated through the greenhouse.

In 1999, an additional set of treatments was added. Basil was seeded in cubes as above, and also in Oasis wedges (BFG Supply, Burton, OH). Oasis wedges ( $2.5 \times 2.5 \times 4.5$  cm (tall)) are composed of the same material as cubes, but fit into trays where the cells are physically separated by plastic (Fig. 2). Trays for Oasis



Fig. 1. Oasis medium was used for seed basil. Fifteen seeds were placed in each cell of Oasis cubes (top) or wedges (bottom).



Fig. 2. Basil plants treated (outer two plants) with *F. oxysporum* strain CS-20 or water (inner two plants). There were significantly fewer plants killed by *Fusarium* wilt in the CS-20 treatment.

wedges have the same perimeter dimensions as cubes, but the wedges hold 102 cells/tray compared to 276 for the cubes. Cells of cubes or wedges were treated with water or CS-20 as above with five replicate trays per treatment. Cubes or wedges were placed in troughs as above. In 1999, the wedge treatments were discarded by the grower at the seedling stage because they could not be bottom-watered effectively using the system designed for the cubes.

In both years, 7 weeks after the plants were placed in the troughs, the number of dead stems and symptomless plants per cube were recorded for ten cubes in each of the five troughs. Troughs were considered as blocks and cubes were considered as replicates. In 1998, the number of plants with wilt symptoms was also recorded. In 1999, even the fresh weight of the plant tops was recorded. Data were analyzed by analysis of variance (SAS, Cary, NC). Data were not transformed.

### 2.3. Pathogen spread through seeding medium

An experiment was conducted to determine if Oasis wedges would slow the spread of the pathogen relative to cubes. Oasis cubes and wedges were seeded as in the previous experiment. The experiment had four treatments: all combinations of cube or wedge, inoculated with *F. oxysporum basilici* Fo-B1 or not inoculated. The cube or wedge closest to the center of each tray was inoculated with 1 ml of  $10^5$  propagules of the pathogen or an equivalent volume of water. After 1 and 2 weeks, three trays (replicates) were sampled for each treatment. Half of each tray was sampled and different trays were used for the two sampling dates. Each cube or wedge was comminuted with 20 ml water for 30 s in a Sorval blender. Ten-fold dilutions of this suspension were placed onto Komada's medium. After 7 days, the number of cfu was recorded. Data were log-transformed before analysis by linear regression in each of the three directions radiating from the cell that was inoculated with the pathogen (SAS, Cary, NC). The three directions were considered as subsamples of the replicate (tray). Since only half of each tray was sampled, there

were no data for the fourth direction. There were three replicate trays for each treatment for each sampling time and the experiment was repeated once.

## 3. Results

### 3.1. Biological control experiments

In both years, at 7 weeks after the plants were placed in the troughs, CS-20 significantly ( $P \leq 0.01$ ) reduced the number of dead stems/cube, with an average of 2.0 nontreated and 0.96 treated dead stems/cube in 1998 and 6.7 nontreated and 5.7 treated in 1999 (Table 1, Fig. 2). In 1998, treated cubes had significantly ( $P \leq 0.03$ ) fewer stems with wilt symptoms (0.28/cube) than nontreated ones (0.76). There was a trend ( $P \leq 0.10$ ) for higher top fresh weight per cube for plants treated with CS-20 (62.64 g) compared to those not treated (60.11 g) in 1999.

### 3.2. Pathogen spread through seeding medium

For the two treatments inoculated with the pathogen, the number of cfu recovered decreased significantly as the distance from the inoculation site increased ( $P \leq 0.0001$  for both the cube and the wedge) (Fig. 3, Table 2). The number of propagules recovered from the noninoculated treatments did not change as the distance increased from the cell inoculated with water ( $P \leq 0.60$  for cube,  $P$  not calculated for wedge due to the high proportion of zeroes).

There were significantly more cfu recovered from cubes inoculated with the pathogen than from wedges inoculated with the pathogen ( $P \leq 0.0001$ ; Table 2). In the cube system, at two weeks after inoculation, the pathogen was recovered 13 cm away from the inoculation site at populations significantly higher ( $P \leq 0.05$ ) than background levels (up to  $10^6$  propagules/cube). Because this pathogen is seedborne and because of the inability to distinguish other naturally occurring *F. oxysporum* from the inoculated pathogen,

Table 1  
Effect of *F. oxysporum* strain CS-20 on symptoms of *Fusarium wilt* and top fresh weight of basil

Treatment	Year			
	1998		1999	
	Mean number of dead stems cube <sup>-1</sup>	Mean number of wilted plants cube <sup>-1</sup>	Mean number of wilted plants cube <sup>-1</sup>	Mean top fresh weight cube <sup>-1</sup>
Control	2.00	0.76	6.66	60.11
CS-20	0.96	0.28	5.66	62.64
<i>F</i> -value	1.88	4.71	20.99	2.71
d.f.	9	1	1	1
<i>P</i> ≤	0.01	0.03	0.01	0.10

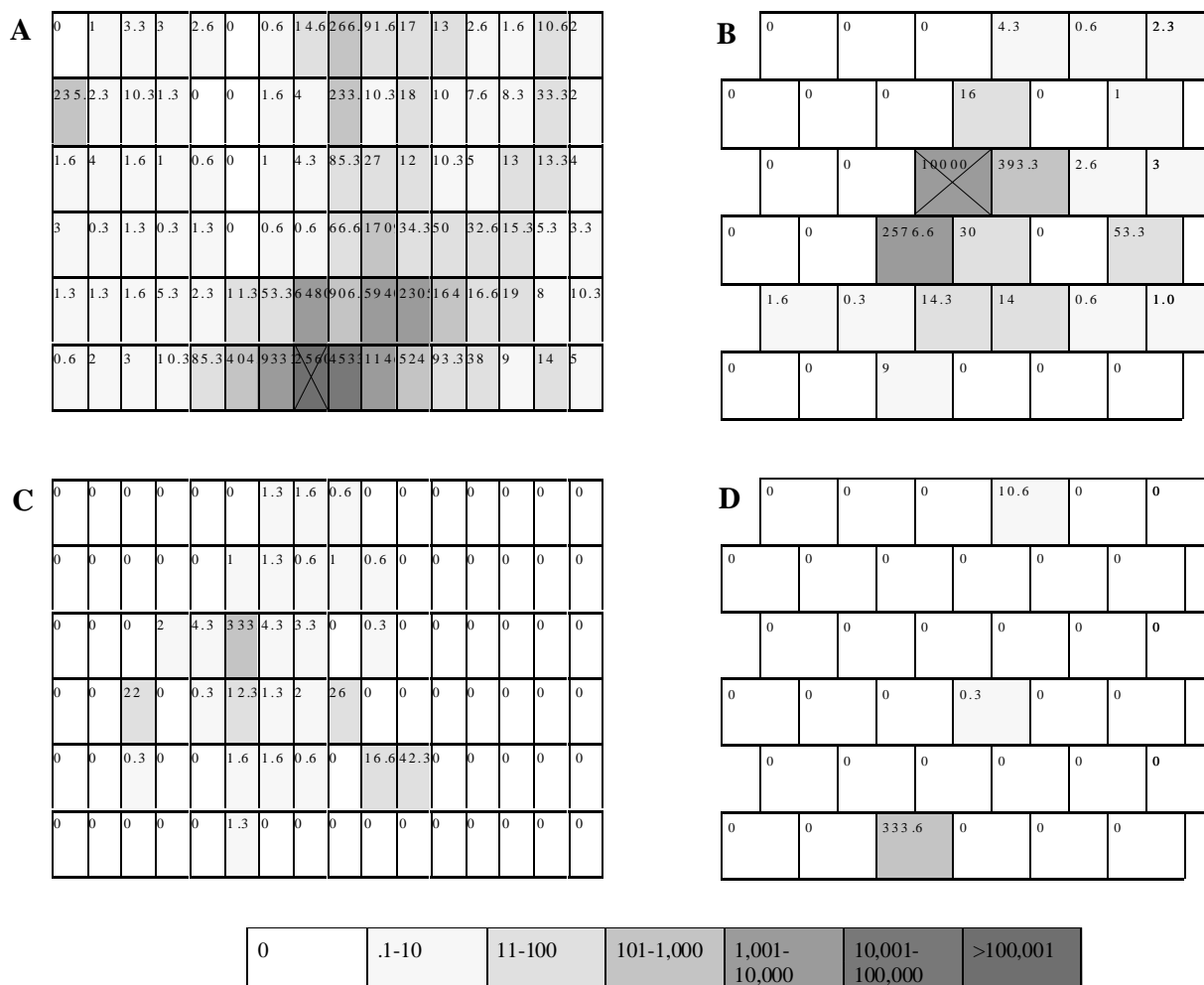


Fig. 3. Spread of *F. oxysporum* f. sp. *basilici* after two weeks in Oasis cubes or wedges. (A) cubes inoculated with the pathogen at the square marked "X", (B) wedges inoculated with the pathogen at the square marked "X", (C) cubes not inoculated, and (D) wedges not inoculated. Fifteen basil seeds were planted in each cell and the cell nearest to the center of the tray was inoculated with either water or 1 ml of a  $10^5$  aqueous suspension of the pathogen. Because only half of the tray was sampled, only half of the tray is shown. Pathogen was present in the noninoculated treatments due to naturally infested basil seed.

Table 2

Effect of type of seeding medium, and distance and direction from the point of inoculation on the population of *F. oxysporum* in the seeding medium

Source	Degrees of freedom	F-value	Pr > F
Distance from inoculation site	1	172.36	0.0001
Type of seeding medium	1	7.07	0.0100
Inoculum (present or absent)	1	30.24	0.0001
Direction from inoculation site	2	3.39	0.0403
Replicate	2	0.28	0.6108

Data are from one representative experiment.

the population size of *F. oxysporum* recovered from the noninoculated controls was considered the background level of this fungus.

#### 4. Discussion

*F. oxysporum* strain CS-20 was selected for its ability to control Fusarium wilt on tomato, caused by *F. oxysporum* f. sp. *lycopersici*. Data presented here demonstrate the potential of strain CS-20 to reduce losses in basil caused by *F. oxysporum* f. sp. *basilici*. Because the primary mechanism of strain CS-20 is induced resistance (Larkin and Fravel, 1999a), it is possible that strain CS-20 might be host-specific in controlling Fusarium wilt. However, in addition to tomato, strain CS-20 reduced losses due to Fusarium wilt in unrelated plants that included muskmelon (Larkin and Fravel, 1999c) and, from data presented here, in basil also.

From tests to compare the cubes to the wedges for pathogen dispersal, cubes were more conducive to increasing pathogen populations than were the wedges. While the pathogen was detected in the circulating

nutrient solution, and others have found the pathogen on surfaces throughout greenhouses (Rekah et al., 2000), spread from infected seed during the initial growth stages is likely to be an additional source of pathogen dispersal. From our results, starting plants in a medium where plants are physically separated has the potential to reduce the spread of the pathogen at this stage of growth. The fact that the grower discarded the treatments with the Oasis wedges reminds us that growers have many factors in addition to disease problems to consider in an overall production system. What makes the most sense from a disease control standpoint may not fit in with the production system for other reasons.

In our tests, strain CS-20 did not completely suppress the disease. It is possible that with additional research, the level of control could be improved. Currently, strain CS-20 should be considered as an additional tool for disease management to be used in conjunction with other methods such as sanitation and resistant cultivars (Reuveni et al., 1998).

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## References

- Alabouvette, C., Schippers, B., Lemanceau, P., Bakker, P.A.H.M., 1998. Biological control of *Fusarium* wilts. In: Boland, G.J., Kuykendall, L.D. (Eds.), *Plant-microbe Interactions and Biological Control*. Marcel Dekker, New York, pp. 15–36.
- Elmer, W.H., Wick, R.L., Haviland, P., 1994. Vegetative compatibility among *Fusarium oxysporum* f. sp. *basilicum* isolates recovered from basil seed and infected plants. *Plant Dis.* 78, 789–791.
- Farr, D.F., Bills, G.F., Churmur, G.P., Rossman, A.Y., 1989. *Fungi on Plants and Products in the United States*. APS Press, St. Paul, MN, 1252pp.
- Gamliel, A., Katan, T., Yunis, H., Katan, J., 1996. *Fusarium* wilt and crown rot of sweet basil: involvement of soilborne and airborne inoculum. *Phytopathology* 86, 56–62.
- Hebbbar, K.P., Lewis, J.A., Poch, S.M., Lumsden, R.D., 1996. Agricultural by-products as substrates for growth, conidiation, and chlamydospore formation by a potential mycoherbicide, *Fusarium oxysporum* strain EN-4. *Biocontrol Sci. Technol.* 6, 263–275.
- Komada, H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8, 115–125.
- Larkin, R.P., Fravel, D.R., 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. *Plant Dis.* 82, 1022–1028.
- Larkin, R.P., Fravel, D.R., 1999a. Mechanisms of action and dose-response relationships governing biological control of *Fusarium* wilt of tomato by nonpathogenic *Fusarium* spp. *Phytopathology* 89, 1152–1161.
- Larkin, R.P., Fravel, D.R., 1999b. Field efficacy of selected nonpathogenic *Fusarium* spp. and other biocontrol agents for the control of *Fusarium* wilt of tomato. *Biol. Cult. Tests* 14, 116.
- Larkin, R.P., Fravel, D.R., 1999c. Field efficacy of selected nonpathogenic *Fusarium* spp. and other biocontrol agents for the management of *Fusarium* wilt of muskmelon. *Biol. Cult. Tests* 14, 161.
- Rekah, Y., Shtienberg, D., Katan, J., 2000. Disease development following infection of tomato and basil foliage by airborne conidia of the pathogens *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *basilici*. *Phytopathology* 90, 1322–1329.
- Reuveni, R., Dudai, N., Putievski, E., 1998. NUFAR: a sweet basil cultivar resistant to *Fusarium* wilt. *HortScience* 33, 159.
- Wick, R.L., Haviland, P., 1992. Occurrence of *Fusarium* wilt of basil in the United States. *Plant Dis.* 76, 323.